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The wide world of coacervates: from the sea to neurodegeneration

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Abstract

The formation of immiscible liquid phases or coacervates is a phenomenon widely observed in biology. Marine organisms, for instance, use liquid-liquid phase separation (LLPS) as the precursor phase to form various fibrillar or crustaceous materials that are essential for surface adhesion. More recently, it has been realized the importance of LLPS in compartmentalizing living cells and obtaining ordered but dynamic partitions which can be reversed according to necessity. Here, we compare the properties, features, and peculiarities of intracellular and extracellular coacervates, drawing parallels and learning from the differences. A more general view of the phenomenon may in the future inform new studies to allow a better comprehension of its laws.

The role of coacervates in biology

Coacervates are small liquid droplets of two immiscible liquid phases, often caused by the encounter of macromolecules with opposite charges or, sometimes, from the association of hydrophobic proteins. Conventionally, the process of coacervation is divided into two groups: simple and complex coacervates. Simple coacervation occurs when only one macromolecule is involved [1]. Complex coacervation is instead mainly induced by the interaction of polyelectrolytes with opposite charges either in solution or forming a colloidal phase [2]. This phenomenon leads to a polyelectrolyte dense phase (the proper coacervate) and to a coexisting dilute phase. The phenomenon was first observed by Tiebackx who, when studying mixtures of gum Arabic (or acacia gum) and gelatin, found insoluble gelatin-gum Arabic complexes [3].

Coacervates occupy an important position in modern science and are found in several important biological processes, including surface adhesion, cellular compartmentalization, self-assembly, vesicle formation, and cell replication [4–6]. They also play an important role in fields as diverse as food industry, cellular biology, biophysics, and biomaterials [7]. This diversity is reflected by their extremely different compositions and topologies. It has been known for a long time that marine species, such as mussels and **polychaetes** [8] (see glossary), are amongst the organisms that heavily rely on extracellular coacervation for their primary functions. More recently, coacervates have also been associated with intracellular **protein aggregation** and nuclear-pore trafficking, as well as in **neurodegenerative diseases** [5,9].

In this review, we first introduce the role that coacervates may have played in the origin of life, and then describe the forces regulating their formation. Through examples, we then draw parallels between marine extracellular coacervates and those found in human cells and how they relate to neurodegeneration. Throughout, we will explore the commonalities and differences between phase transitions occurring in different species. While not aiming at having an exhaustive coverage of the field, we would like to demonstrate how much can be learned by transversally transferring concepts across different disciplines. It will, for instance, be interesting to assess whether some of the important factors responsible for extracellular coacervate formation (e.g. pH, confinement, solvent composition, and post-translational modifications) play a similar role also in

intracellular coacervation, and vice versa A wider perspective can only help to clarify both fields.

LLPS at the origin of life

Coacervation originates from **liquid-liquid phase separation** (**LLPS**). The importance of this phenomenon is not appreciated for the first time now in the history of Biology; one of the first hypotheses on the origin of life on Earth was formulated by A. I. Oparin who summarized his ideas in a famous book, titled *The Origin of Life* [10]. The central argument of this book was that life might have originated inside coacervates containing myriads of different organic molecules. Oparin observed that coacervates, intended as small droplets of high concentrations of organic molecules, often form autonomously even in dilute solutions. He therefore suggested that oacervation could have been the mechanism through which a fluid phase would separate within the "primordial soup".

Despite the influence that Oparin's work has had on many studies on the origin of life, the importance of coacervates declined rapidly, mainly because it seemed in stark contrast with the presence of well-defined membranes that separate cells from the outside world, as well as those that separate the cellular interior into **organelles** (**Figure 1A**). It is only over the last few decades that evidence accumulated observing the existence of organelles not enclosed in membranes [4], so much so that **membraneless organelles** are now considered essential components of eukaryotic cells [5,9,11]. They have been shown to constitute a more dynamic way to sequester (some time temporarily and reversibly) cellular components from the rest of the cell (**Figure 1B**). Membrane and membraneless organelles can be respectively assimilated by analogy to a grape (membrane organelle) that encloses its seeds and to oil droplets in an aqueous solution (membraneless organelles) (**Figure 1C, D**).

These findings have thus renewed broad interest in Oparin's proposal [10] and have led to new experimental efforts to address the origin of life. For instance, in 2019, Jia et al. started with prebiotically available α-hydroxy acids and prepared polyester droplets that would segregate proteins and **RNA** in a fashion compatible with origin of life conditions [12]. More recently, it was also shown that phase separation may help transforming abiotic ornithine residues into arginines, thus allowing the formation of a dsDNA-binding protein [13].

Which are the forces driving coacervate formation?

Coacervation involves a phase transition, which can lead to LLPS, gelation, aggregation, and/or crystallization depending on the conditions of temperature, salt concentration, pH, crowding, confinement, and protein concentrations [4]. Simple and complex coacervations are, in principle, quite distinct phenomena. Simple coacervates involve only one macromolecule, often a protein [1]. Accordingly, the forces promoting single coacervate formation are essentially those dictating protein aggregation (**Figure 2A**): self-assembly of molecules of the same protein occurs through a combination of electrostatic, hydrophobic, and van der Waals forces. Also essential for simple coacervate and aggregation formation is the protein concentration and the supersaturation point of the protein (in chemistry this is called the solubility product constant) [14]. Under this definition, **amyloid aggregates**, whose formation is usually driven by hydrophobic forces, can be considered as the irreversible end point of simple coacervation [15]. Notable examples of simple coacervate formation and protein aggregation include the formation of Aβ amyloid fibres (*vide infra*) [16] and the excretion of extracellular coacervates from marine animals for **surface adhesion** [17].

On the contrary, formation of complex coacervates appears to be predominantly driven by electrostatic forces between macromolecules of different types, notably **polyelectrolytes** of opposite charges (**Figure 2B**) [2]. Electrostatic neutralization indeed favours LLPS and complex coacervate formation from equilibrium solutions, initially forming droplets that may coalesce, leading to a denser bulk phase or larger droplets which retain both water and salt, in equilibrium with a less dense supernatant depleted of macromolecules [2,7,18,19]. Complex coacervation typically occurs under conditions of electroneutrality, roughly when a 1:1 ratio of polycations to polyanions is achieved. When the two molecule types neutralize each other, they lose their solubility and produce a salting out. If the coacervate is formed solely by proteins, the driving force for coacervation results from specific exposed charged patches on the protein surface. Consequently, the parameters that will determine coacervation are the isoelectric points (pIs) of the protein, that is the pH values at which the proteins do not have net charge, and the pH of the solution. Ionic strength is another important variable: the presence of salt may favour charge compensation and polyelectrolyte partitioning among the coacervate compartment itself and the supernatant and lead, for some systems, to transitions from a

solid precipitate to a liquid coacervate [2]. However, a solution with high ionic strength may, in some cases, inhibit coacervate formation by strongly shielding charges [2]. Finally, there has been a renewed interest in the role of forces involving π-π interactions in complex coacervation, particularly in the framework of partially disordered proteins [20]. These forces are generally associated with interactions between the aromatic rings of Tyr, Phe, Trp, and His, but since π orbitals are also present in peptide bonds and sidechain groups of Gln, Asn, Glu, Asp, and Arg, these residues can potentially contribute to π-π stacking.

Besides the individual contributions of these forces, all variables influencing coacervate formation may also influence the formation of solid precipitates. Their variations can lead to phase transitions from monodisperse species to solid precipitates, from precipitates to liquid coacervates, and back to a monodispersed solution. Indeed, recent work has confirmed the role of charge density, hydrogen bonding, and polyelectrolyte strength [21–24] in these processes, but overall demonstrated that entropy dominates coacervation while enthalpic contributions are negligible [25,26].

In vitro, complex coacervates have been formed using several different macromolecules, such as polysaccharides [27], polyelectrolytes [28], peptides [29], and nucleic acids [30]. Intrinsically disordered proteins or proteins containing disordered domains are particularly prone to complex coacervation [5] (**Figure 2B**). *In vivo*, it has been shown that, if the macromolecule concentration exceeds for any reason (over-production, reduced protein clearance, etc.) its solubility and thus the solution becomes supersaturate, the coacervate can precipitate. Classic examples include the mixing of histones that are rich in basic residues with negatively charged proteins or the mixing of RNA with short cationic peptides or two oppositely charged proteins [31].

We could then wonder whether these forces determine a specific structure of coacervates: it is not, by definition, possible to speak of an intrinsic common structure because we are at least initially dealing with liquid phases which may proceed to completely different end-points. There have nevertheless been attempts to capture three-dimensional structural elements by cryo-electron microscopy (cryo-EM) methods. For example, a distinct “sponge structure” was described in droplets by cryogenic temperature high-resolution scanning EM (cryo-HRSEM) [32]. More recently, Kizilay et al. concluded

that cryo-transmission EM (TEM) images of coacervates indicate that they form subunits organized at large length scales within dense and dilute coacervate domains [33].

Coacervates of marine origin: a lesson from the sea

Why do marine organisms produce extracellular coacervates? Research has shown that these organisms often produce coacervates to solve the problem of achieving and maintaining strong adhesion on polar surfaces underwater (surface adhesion). Instead of secreting highly soluble polyelectrolytes directly into seawater where these molecules would be quickly diluted by diffusion, marine organisms secrete various types of biopolymers or aqueous mixtures of polyelectrolytes which undergo LLPS mainly to facilitate adhesion, positioning, and spreading [30].

The instructive example of coacervates from mussels and polychaetes

Possibly the best studied marine organisms from the point of view of extracellular coacervate formation are mussels. To anchor to surfaces, mussels produce the byssus, i.e. a bundle of proteic threads protruding from the base of an internal organ called the foot (**Figure 3A**) [32]. This organ produces the byssus in its ventral groove that starts from the so called distal depression (**Figure 3B**) and ends with an adhesive plaque, shaped like a spatula, at the tip of the thread attached to the external surface. Precursor proteins and a variety of chemicals are injected in the cavity generated by the distal depression (**Box1** and **Figure 3C**) from three gland reservoirs, the phenol, collagen and accessory glands [33-35]. This mixture of molecules then form a coacervate (**Figure 3D**) and move along the ventral groove up to the plaque. The coacervate evolves into a fiber and the plaque becomes an integral part of the byssus thread. Although strictly speaking, coacervates originating from polyelectrolites of the same charge should be classified as “simple” coacervate, in cases like that of mussels, whose adhesive proteins are mainly positively charged polyelectrolytes [30], the multiplicity of precursor proteins, optimization of pH and the addition of several other molecules make these coacervates behave as complex ones.

In the *Mytilus* and *Perna* genera of mussels, for instance, the plaque, that is the terminal part of the byssus, contains an assembly of several collagenous materials and an ensemble of tyrosine-rich proteins, all coming from the three glands of the foot. The main precursor proteins involved are called mussel foot proteins 2 to 6 (mfp-2 to mfp-6). In

addition, there is an accessory protein (commonly dubbed mfp-1) [31] which enhances the mechanical properties of the byssus by means of Fe^{3+} cross-links [36]. All mfps are eventually modified with post-translational transformation of tyrosines into 3,4-dihydroxyphenyl-L-alanine (L-**DOPA**) residues, a reaction catalysed by the tyrosine hydroxylase enzyme [8,37]. Notably, L-DOPA is the precursor of the cathecolamine neurotransmitters dopamine, noradrenaline and adrenaline in the nervous system. L-DOPA can interact through formation of covalent interactions and coordination complexes and is a key molecule for adhesion in wet environments. Oxidation of DOPA to DOPA-quinone by a cathecholoxidase enzyme or non-enzymatic means guarantees the cross-linking that permits strong adhesion to substrates [38]. Proteins containing L-DOPA-modified residues have thus unique adhesion properties that are exploited by marine animals for their purpose.

Sabellariidae such as the sandcastle and the honeycomb worms *Phragmatopoma californica* and *Sabellaria alveolata* represent a marine metazoan family presenting complex coacervation. In these polychaetes, coacervates are found in the tube mucous that these animals utilize for building their tube, cementing it with solid particles dispersed in the external environment (i.e. sand grains, bits of seashells, feces, etc.) [39]. Three precursor proteins involved in the adhesion process, Pc1, Pc2, Pc3, were isolated from *P. californica* [40]. Pc1-2 are characterized by repeats of positively charged motifs, mainly rich in Gly, Lys and DOPA residues [41]. Pc3 is composed of 4-13 Ser residues separated by single Tyr residues [42]. As in mussels, phase coacervation in *P. californica* is pH dependent. It occurs in the cement glands at a pH comprised between 5 and that of sea water which strongly depends on the CO_2 content of the atmosphere but anyway around 8.2 [42]. In these organisms, coacervation involves the precursor basic proteins, an acidic pSer rich protein and Ca^{2+} and Mg^{2+} ions [41]. These components promote phase separation when mixed in an electroneutral ratio (complex coacervation). After water release, the coacervates are secreted in the external environment for permitting tube formation. These examples clearly show the significance of coacervation in marine animals and clarify the factors that determine their formation.

Intra-cellular coacervates

Increasing evidence shows that LLPS underlies the formation of membraneless organelles inside cells [34]. The list of cell compartments that are thought to be formed through LLPS grows rapidly and covers an incredibly diverse number of cellular functions. Classic examples widely studied are the germ cell P-granules of *Caenorhabditis elegans* embryos [35], the polar granules of *Drosophila melanogaster* embryos [36], the stress granules that appear in cultured yeast and mammalian cells under different forms of metabolic stress such as nutrient deprivation [37,38], the neuronal granules [39,40], the nucleolus [41,42], and the ribonucleoprotein (RNP) assemblies [43] (**Figure 4A**).

These organelles constitute a dynamic organization principle that allows cellular compartmentalization and creates an infrastructure while still permitting internal rearrangements and regulating entrance in liquid droplets [44]. Their confined nature also favours the increase of reaction rates of various cellular processes by increasing local concentrations by as much as two orders of magnitude. The consistency of these organelles covers a continuum from more liquid /gel-like species to more fibrillar-like ones, depending on the strength of the interactions among the constituents and depending on structural, functional, or organizational needs. Paradigmatic examples of the various extremes are the gel-like formation of the nuclear-pore complex that acts as a barrier to the diffusion of molecules above 30-40 kDa in or out of the nucleus [45–47] and the amyloid fibrils of the Aβ peptide and other aggregation-prone proteins observed in neurodegeneration [16].

Intracellular coacervates or membraneless organelles have been observed in a wide spectrum of cell types. The molecular composition of these granules has been extensively analysed. They are typically granules which contain proteins and RNA. One of the common features of the proteins involved is the presence of multivalent binding, prion-like, or intrinsically disordered domains which may promote protein-protein interactions in various manners [48]. Several of the proteins are also aggregation prone and, when they carry disease-causing mutations, they can often form amyloid fibres [49,50]. They can also promote a transition from a liquid droplet to a solid phase *in vitro*, leading to the hypothesis that a liquid-to-solid phase transition is a mechanism of cellular toxicity [51]. A role of RNA binding in LLPS is also evident: in humans, there are 240 genes that encode proteins with prion-like domains [52]. Of these, 72 are RNA binding proteins, among which are FUS, TDP-43, TAF15, EWSR1, hnRNPA1, hnRNPA2, and TIA-1; these are all

components of ribonuclear protein (RNP) granules and heavily implicated in neurodegenerative diseases [53]. For many of the RNA binding proteins, solutions containing highly purified proteins are able to undergo LLPS *in vitro* [6,51,54,55] having this ability mediated by the intrinsically-disordered regions [56,57].

Coacervates in neurodegeneration

Besides being involved in non-pathologic events, intracellular coacervates are also associated with several neurodegenerative diseases even though their precise causal significance is still debated. For example, coacervation appears to be the basis of LLPS droplet formation that would occur before developing insoluble amyloid aggregates (**Box 2** and **Figure 4B**) [16]. It is thus possible that amyloid aggregates represent an extreme end stage of the process of phase separation which in some cases cannot be reversed back to its normal dynamic state.

In addition, the field of disease-associated intracellular LLPS originally developed from studies focused on proteins involved in **amyotrophic lateral sclerosis** (**ALS**) and **Frontotemporal dementia (FTD)** and their relationship with stress granules [58]. Amongst the ALS/FTD-related proteins are FUS, TDP-43, hnRNPA1, and TIA-1 [59]. These are the same RNA binding proteins found in RNP granules. Through studying these condensates, appreciable new insights were gained into the molecular bases of disease. Most people now consider the coacervates of these proteins as the necessary species whose function is that of binding and trapping crucial RNA sequences [60,61]. It was also observed that stress granule proteins form dynamic liquid droplets that mature to form solid aggregates through an aberrant liquid-to-solid phase transition [6]. Mutations observed in these proteins of ALS/FTD patients may accelerate this transition [52]. Therefore, it was suggested that stress granules could be the sites for disease biogenesis even though most of the proteins involved are highly aggregation-prone also outside these condensates [37,38].

A different but particularly interesting family of proteins also involved in ALS/FTD are the arginine-rich proteins containing proline-arginine and glycine-arginine dipeptide-repeats [62]. These poly-peptides, which contain long chains of uninterrupted tandem repeats, are produced by repeat-associated non-ATG translation of the ALS/FTD-causing G4C2 repeat expansion of *C9orf72* [63,64]. Dipeptide-repeat proteins have intrinsic

aggregation-prone properties and accelerate aberrant phase transitions of other RNA binding proteins. Many more proteins have now been associated to disease-related LLPS phenomena.

Similarities and differences between intra- and extra-cellular coacervation

There can be no doubt that coacervates and the process of LLPS are everywhere in Nature. Consequently, their formation has widely been studied from several points of view. It is however only relatively recently that we have realized the importance of this process in constituting a flexible and dynamic way to form intra-cellular membraneless organelles which have all the features of oil droplets into water. Here, we have compared intra-cellular and extra-cellular coacervates. While the physical forces and the main principles remain the same in both cases, several interesting differences and commonalities can be found. Coacervates from marine organisms are mostly transient states that evolve to create new macroscopic structures, such as the byssus in mussels and the tube in polychaetes. The process bears impressive analogies with what happens in intracellular LLPS: it starts with weak and reversible interactions in a confined environment which can in principle take several different routes. For example, maturation from the coacervate into amyloids is thought to occur in specific and, in a certain sense, extreme cases which are those that lead to amyloid formation, toxicity and neurodegeneration (**Box 2 and Figures 3, 4**).

A noticeable difference between the two processes is that most extracellular coacervation in marine animals requires a single component, whereas intracellular coacervation may involve both simple and complex coacervates. Another constant in intracellular LLPS formation is the importance of RNA in the process. The functions of several membraneless organelles are in fact strongly intertwined with RNA, such as occurs for mRNA storage in stress granules, mRNA decay in P-bodies, mRNA splicing in nuclear speckles, and rRNA synthesis in nucleoli [61]. A role of RNA does not however come as a surprise since complex coacervation is often triggered by the co-presence of two oppositely charged polymers. Indeed, RNA acts as a potent, biologically important nucleator of intra-cellular phase separation [40].

Another interesting peculiarity is the presence of L-DOPA in marine organisms. This post-translational modification is likely under the control of tyrosine hydroxylase, the rate-limiting enzyme that catalyzes hydroxylation of tyrosine to L-DOPA [65]. This is the

precursor of the dopamine, noradrenaline, and adrenaline neurotransmitters. The role of L-DOPA in marine organisms is thought to be that of enhancing adhesion properties, although we have recently demonstrated that the Pvfp-5β protein from mussels retains its adhesion properties also in its non-modified form [66]. While no account of DOPA-modified intracellular proteins is currently available, it is tempting to speculate that this post-translational modification could, in the future, be found to have a role also in intracellular coacervates and perhaps be directly associated with pathology. This is not necessarily a wild flight: as an important neurotransmitter, DOPA plays, for instance, a key role in Parkinson disease in which specific reduction of dopamine, the derivative of DOPA, is found in certain vulnerable cells such as Substantia Nigra (SN) [67]. Parkinson disease is genetically and molecularly associated to a phase transition to amyloid species mediated by alpha-synuclein [68]. It is thus tempting to speculate that L-DOPA as treatment for Parkinson could affect the alpha-synuclein phase transition.

Finally, it is interesting to acknowledge the duality of the life/death nature of the coacervates considered in this study. They can be at the same time an important feature that permits underwater life in metazoans or give origin to membraneless organelles as nucleoli, Cajal bodies, germ P-, and polar granules. On the other hand, intracellular coacervates can have negative effects and lead to cellular degeneration as it happens in stress granules observed, for instance, in ALS/FTD patients. It remains for us to understand how this double perspective is regulated in Nature and the specific physical laws that lead to modulate the processes involved.

Concluding remarks

In conclusion, it is fair to notice that, although being two aspects of the same phenomenon, the worlds of intra- and extracellular LLPS constitute at the moment watertight compartments with no exchange between the two fields. Marine coacervates have mainly attracted large interest for their potential as new biomaterials and for wider applications in biotechnology, often neglecting a more general framework of the processes involved. Intracellular coacervation is instead an increasingly emerging field of primary relevance for basic biology and medical implications. It will be interesting to see whether the two fields can merge in the future. A more detailed comparison could allow us to gain a wider picture of the physical laws that inform this fascinating and important biological

phenomena (see **Outstanding questions**). It will be of specific interest to scrutinize more in details the role of DOPA or other similar post-translational modifications in coacervation. The knowledge gained might also have unanticipated implications for completely different disciplines.

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Figure Legends

Figure 1. Comparison between membrane and membraneless organelles. A) A) Immunostaining of eukaryotic cells (HEK293) with stained nuclei (blue, DAPI) and mitochondria (green, frataxin antibody) (Vannocci, unpublished data). B) A coacervate of the protein FUS fused to GFP (Ruepp et al., unpublished data). C) As an analogy, membrane organelles resemble a grape which encloses its well separated seeds. D) In contrast, membraneless organelles resemble droplets of oil in a aqueous solution.

Figure 2. Simple and complex coacervate formation. A) The forces involved in coacervation. Hydrophobic forces are mainly responsible for simple coacervation, whereas complex coacervation is mainly driven by electrostatics. Other types of interactions may contribute. B) Examples of the molecules considered more prone to give LLPS.

Figure 3. Schematic drawing of byssus formation in a mussel. A) Mussel with byssus fibers protruding and the foot touching the external surface, in preparation of a the formation of a new thread of byssus fibre. B) Detail of the foot-surface interaction with the formation of a distal depression. C) Glands of the foot deliver Mfp proteins to the cavity and adjust pH and redox conditions. D) A coacervate containing a mixture of electrolytes and other molecules undergoes a LLPS and eventually evolves into a solid foam and a byssus.

Figure 4. Coacervate formation and aggregation of Aβ peptides. A) A schematic eukaryotic cell showing some of the most relevant membraneless organelles, both inside and outside the nucleus. B) The pathway that leads to Aβ starts with amyloid precursor protein (APP internalization through a specialized clathrin- mediated endocytic pathway. After internalization, APP is sorted in early endosomes where the low pH favours β- secretase cleavage which produces soluble fragments The C- terminal fragment, β- CTF, is then further cleaved by γ- secretase to produce Aβ. This peptide moves to the multivesicular bodies which can fuse with the plasma membrane to release intraluminal vesicles as exosomes. APP retrieval from the early endosomes occurs via other non-detailed pathways. Aβ aggregation and fibre formation is promoted by the low pH, the

lipid environment and confinement. B) Cross-β structure of amyloid fibers now well described in Alzheimer disease (PDB code 2rnm).

Glossary 450 words

· Amyloid aggregates: insoluble protein aggregates of fibrillar morphologies that have a β-rich content and a so-called cross-β structure in which the β-sheet assembly runs perpendicular to the fibre axis.

· Amyotrophic lateral sclerosis (ALS) / Frontotemporal dementia (FTD): two distinct progressive neurodegenerative diseases that affects nerve cells in different parts of the brain and the spinal cord. They are correlated by some of the causative proteins involved.

· Cavitation: formation of bubbles within a liquid at low-pressure regions that occur where the liquid has been accelerated to high velocities.

· DOPA: L-3,4-dihydroxyphenylalanine, an amino acid that is part of the biological processes of several animals and plants, including humans. L-DOPA is the precursor to several neurotransmitters collectively known as catecholamines.

· Liquid-liquid phase separation or LLPS: a process in which a homogenous fluid separates into two distinct liquid phases, one more concentrated and one diluted; the process is normally reversible.

· Membranelless organelles: In contrast to organelles with a lipid bilayer membrane, membraneless structures are formed through a process known as liquid-liquid phase separation. Membraneless structures help temporary and dynamic compartmentalization of the cytoplasm, as well as the interior of the nucleus. Examples are nucleoli, Cajal bodies, germ P- and polar granules.

· Neurodegenerative diseases: a heterogeneous group of diseases that are characterized by progressive degeneration of the structure and function of the central nervous system or of the peripheral nervous system. Examples of common neurodegenerative diseases include Alzheimer's disease and Parkinson's disease.

· Polyelectrolytes: polymers in which the units contain an electrolytic group. Typically, polycations containing positive charges and polyanions containing negative charges are polyelectrolytes. Polyelectrolyte share properties of electrolytes and of polymers.

· Polychaetes: a class of anellid worms also known as bristle worms. The 10,000 or so species described in this class live in all temperature conditions, from the coldest temperatures of the abysses to the very high temperatures hydrothermal vents.

· Protein aggregation: the phenomenon in which proteins or peptides self-assemble.

· Organelles: cellular compartments with a specific function. Organelles are generally enclosed within lipid bilayers (membrane-bound organelles) but there are also functional units without a surrounding lipid bilayer (membraneless organelles). Examples of membrane-surrounded organelles are the nucleus and the mitochondria.

· RNA: Ribonucleic acid is a biological macromolecule that covers essential roles in coding, decoding, regulation and expression of genes.

· Surface adhesion: the tendency of dissimilar surfaces to stick to one another.

Boxes 400 words

Box 1 - Byssal formation in mussels

Byssal formation initiates with a temporary attachment of the distal portion of the mussel foot to the selected surface (**Figure 3A**). The main consequence of the initial attachment and the concomitant pressing of the foot against the ceiling of the shell is the production of a negative pressure that forms a cavity (**Figure 3B**)[69]. The resulting confined environment is a reaction chamber (**Figure 3C**) in which the mussel creates conditions of pH and ionic strength different from those of the surrounding seawater (pH range 2–4 and ionic strength of 0.150 M in the **cavitation**, to be compared with pH 8 and ionic strength of 0.7 M in seawater)[70]. This pH difference is obtained by effective proton pumping, probably similar to that reported for stomach acidification by H+/K+-ATPase[71] or, more notably, in lysosomes[72]. Although the actual function of an acidic pH remains unclear, one of the hypotheses is that it helps controlling the coacervation process of the positively charged byssal proteins. Confined cavitation also allows the control of the redox environment protecting the confined environment from the highly oxidizing conditions of oxygen-saturated seawater. This is essential to guarantee the redox state of DOPA to DOPA-quinone which are all equally important for adhesion. The presence of DOPA contributes to maintenance of reducing conditions[73]. It is unclear how long this redox difference persists after foot lift-off, whereupon the plaque equilibrates with ambient seawater oxygen. These conditions allow secretion of DOPA-rich adhesive proteins which undergo condensation as fluid–fluid phase separations leading to coacervate formation (**Figure 3D**)[74]. Although metastable, the coacervates form a transient liquid phase well separated from the aqueous phase and allow underwater adhesion as they are denser than water and can directly attach to a surface without being diluted by diffusion. For the byssus to grow, the process is reiterated over and over again. Coacervates are then thought to solidify by protein cross-linking and contribute to the formation of byssal plaques, but alternative phase transitions such as phase inversion are also possible.

Box 2 – The role of coacervation in Alzheimer disease

The events that produce intra-cellular coacervates are in many ways similar to what happens in mussels. In Alzheimer disease, for instance, the molecule that aggregates and undergoes a conformational change is the beta-amyloid peptide (Aβ); this peptide forms the aggregates found in the cerebral plaques found in Alzheimer patients and is one of the single coacervates best studied. In aqueous solutions, the conformation of the Aβ peptides is random coil (**Figure 4B**). Aβ is produced in early endosomes by a specialized endocytic mechanism[75].

Three lines of evidence support the hypothesis that oligomerization of Aβ occurs in endosomes. First, *in vitro* experiments have suggested a pH dependence of Aβ oligomerization with an optimal pH around 6.0. Indeed, the pH of the early endosomes is 5.5-6.0 (which matches the isoelectric point of Aβ peptide)[76]. These pH values are much lower than those of the cytosol and achieved with mechanisms similar to those observed in lysosomes and in marine coacervates. Second, aggregate formation is favoured by the higher local concentration of Aβ in the confined space of endosomal lumen. Finally, a further element to promote the event is the lipid environment and composition: it was shown that raft lipids formed of neutral sphingolipids and cholesterol activate β-secretase[75]. The multivesicular endosomes contain the ganglioside GM1 which acts as an amyloid seed for Aβ fibrillation. Ganglosides are molecules composed of a glycosphingolipid (the waxy lipid ceramide and an oligosaccharide) and one or more sialic acid groups. These results have suggested that endosomal Aβ could already be a pathogenic specie. pH and lipid environment could play major roles in both phase transition as well as aggregation of such peptides into amyloid conformation and thus be implicated in the aetiology of dementia.

Early studies have shown that endosome-generated Aβ peptides are then released out from the cell via a pathway that involves association with exosomes[75]; upon fusion with the plasma membrane, intraluminal vesicles of multivesicular endosomes are released into the extracellular space as observed in amyloid precursor protein (APP) transgenic mice that exhibit behavioural deficits also in the absence of extracellular plaques[72]. It is interesting to notice how closely the process of Aβ aggregation parallels and resembles that of byssus formation in the role that pH and confinement have in the formation of the final species.

Figure 1

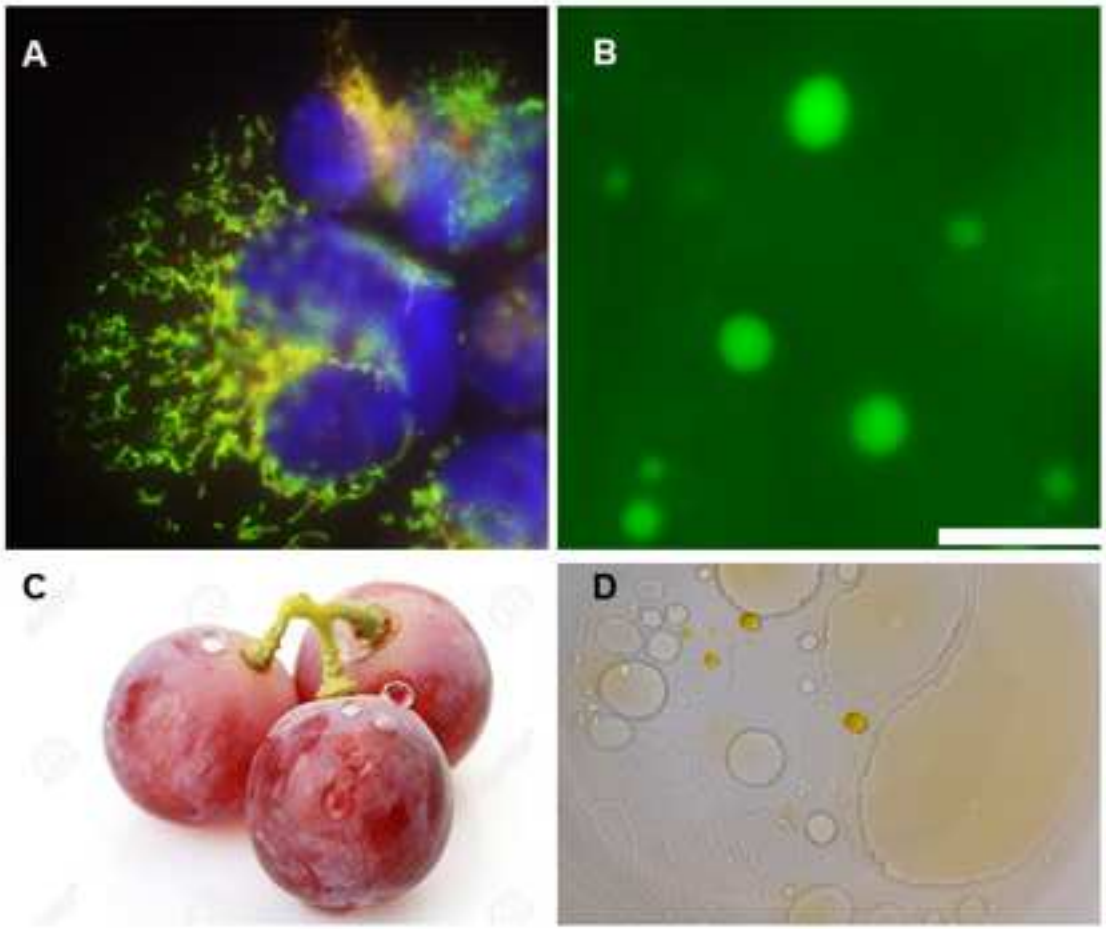
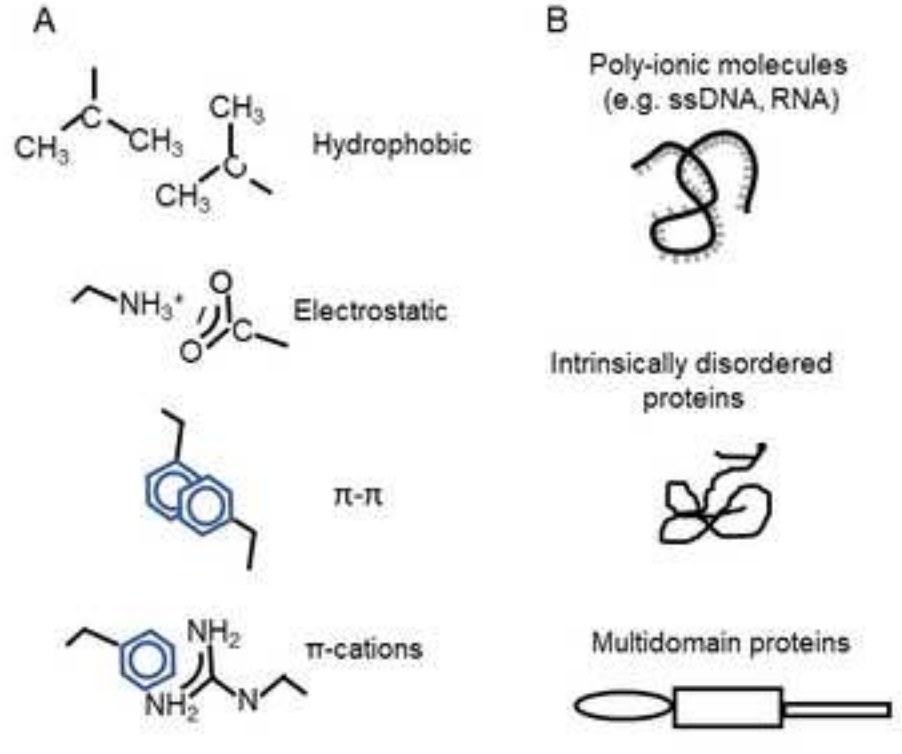


Figure 2



Figure

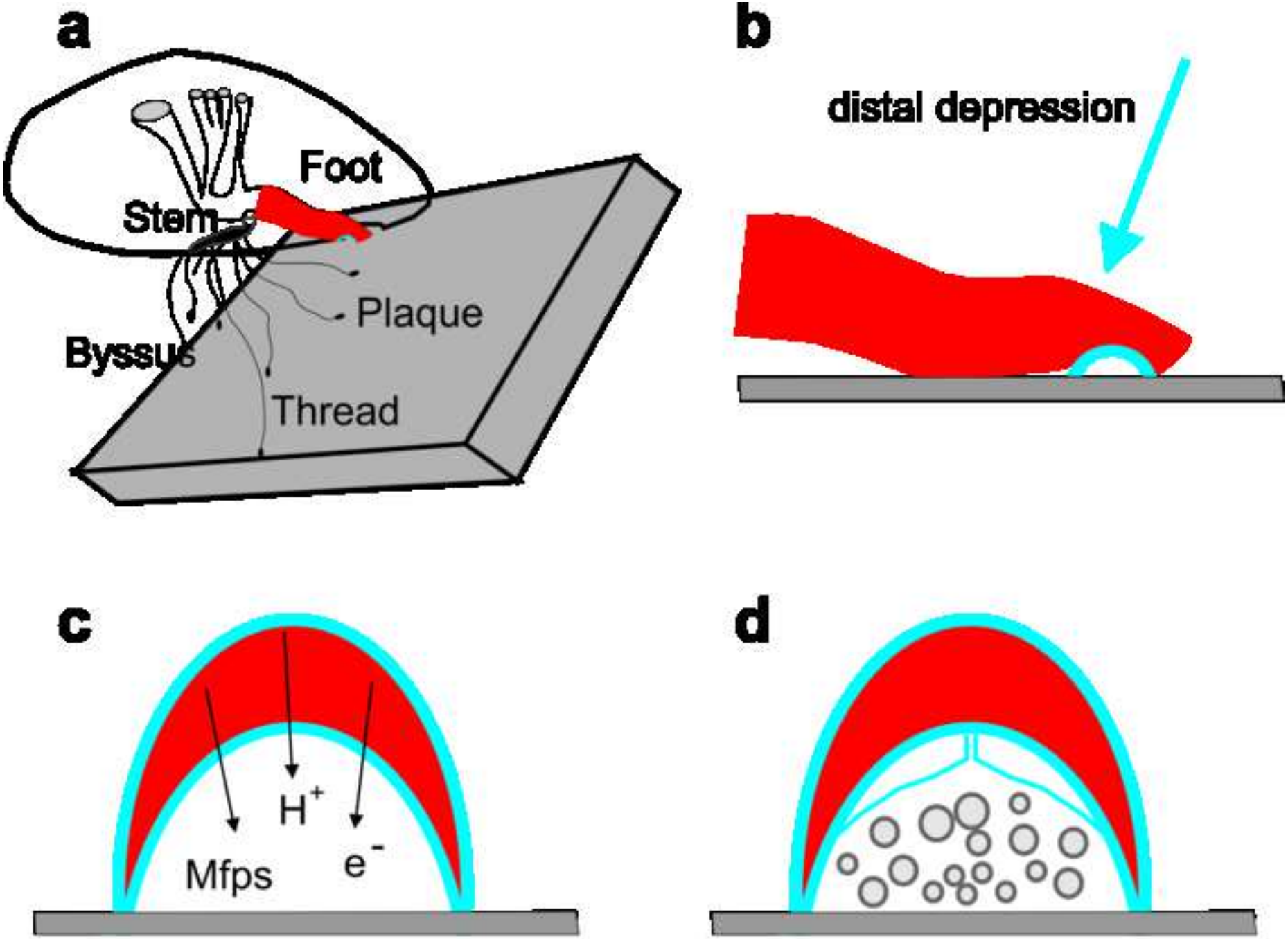


Figure 4